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European Journal of Pharmacology 422 (2001) 83–86

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Short communication

Role of vanilloid VR1 receptor in thermal allodynia and hyperalgesia in diabetic mice

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Received 29 March 2001; received in revised form 10 May 2001; accepted 15 May 2001

Abstract

We examined the role of the vanilloid VR1 receptor in the thermal hyperalgesia and allodynia seen in diabetic mice. Tail-flick latencies at source voltages of 35 and 50 V for a 50-W projection bulb in diabetic mice were significantly shorter than those in non-diabetic mice. Tail-flick latencies at 35 and 50 V in diabetic mice were increased by pretreatment with anti-vanilloid VR1 receptor serum. Intrathecal (i.t.) injection of anti-VR1 serum resulted in a significant increase in the tail-flick latency at 50 V in non-diabetic mice. However, i.t. pretreatment with anti-vanilloid VR1 receptor serum did not affect the tail-flick latency at a heat intensity of 35 V in non-diabetic mice. Thus, it seems likely that thermal allodynia and hyperalgesia in diabetic mice may be due to the sensitization of vanilloid VR1 receptors in primary sensory neurons in the spinal cord.

Keywords: Diabetes; Vanilloid VR1 receptor; Allodynia; Hyperalgesia; Capsaicin; Spinal cord

1. Introduction

Behavioral reactions of hyperalgesia in animal models of diabetes have been described previously (Calcutt and Chaplan, 1997, Kamei et al., 1991; Ohsawa and Kamei, 1999a). We recently reported that the heat intensity at a bulb voltage of 35 V, which did not produce a tail-flick response in non-diabetic mice, produced a tail-flick response in diabetic mice (Ohsawa and Kamei, 1999a,b). Furthermore, the tail-flick latency after heating the tail at 50 V in diabetic mice was significantly shorter than that in non-diabetic mice (Ohsawa and Kamei, 1999a,b). However, there were no significant differences in the tail-flick latencies between diabetic and non-diabetic mice after heating the tail at 25, 65 and 80 V (Ohsawa and Kamei, 1999a,b). Based on these results, we proposed that diabetic mice exhibit thermal allodynia and hyperalgesia in the tail-flick test (Ohsawa and Kamei, 1999a,b). Furthermore, we also suggested that the thermal allodynia and hyperalgesia in diabetic mice may be due to the hyperactivity of C-fiber in the spinal cord, since pretreatment with capsaicin 24 h before testing reverses thermal allodynia and hyperalgesia in diabetic mice (Ohsawa and Kamei, 1999a). The vanilloid VR1 receptor is a ligand-gated, non-selective cation channel that is expressed predominantly by sensory neurons, probably in unmyelinated C-fibers (Caterina et al., 1997, 1999; Helliwell et al., 1998; Tominaga et al., 1998). Vanilloid VR1 receptor can also be activated by capsaicin, noxious heat ($> 43°C$) and extracellular acidification ($pH < 6$) (Caterina et al., 1997; Tominaga et al., 1998), which excite nociceptors and evoke pain in humans or pain-related behaviors in animals (De Castro et al., 1998; Garcia-Hirschfeld et al., 1995; Steen et al., 1992). Thus, these results raise the possibility that vanilloid VR1 receptor might be involved in the mechanisms of thermal allodynia and hyperalgesia in diabetic mice. Therefore, in the present study, we examined the role of vanilloid VR1 receptor in the thermal allodynia and hyperalgesia in diabetic mice using anti-vanilloid VR1 receptor serum.

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01059-7

2. Methods

2.1. Animals

Male ICR mice (Tokyo Laboratory Animals Science, Tokyo, Japan), weighing about 20 g at the beginning of the experiments, were used. They had free access to food and water in an animal room which was maintained at 24 ± 1°C with a 12-h light–dark cycle. Animals were rendered diabetic by an injection of streptozotocin (200 mg/kg, i.v.) prepared in 0.1 N citrate buffer at pH 4.5. Age-matched non-diabetic mice were injected with vehicle alone. The experiments were conducted 2 weeks after the injection of streptozotocin or vehicle. Mice with serum glucose levels above 400 mg/dl were considered diabetic. This study was carried out in accordance with the guide for the care and use of laboratory animals as adopted by the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Sports and Culture.

2.2. Assessment of the nociceptive response

The nociceptive response was evaluated by recording the latency to withdraw the tail in response to different rates of noxious skin heating (Ohsawa and Kamei, 1999a). Briefly, the tails of mice were exposed to a focused beam of light from a 50-W projection bulb. The heat intensity was set to one of two values by adjusting the source voltage of the bulb to 35 and 50 V. When a withdrawal response occurred, the stimulus was terminated and the response latency was measured electronically. In the absence of a response up to a predetermined maximum latency (30 s), the trial was terminated to prevent tissue damage. The heat intensities at 35 and 50 V produced surface skin heating rates of 0.4 and 0.9°C/s, respectively.

2.3. Intrathecal injection

Intrathecal (i.t.) administration was performed following the method described by Hylden and Wilcox (1980). Each i.t. injection was administered using a 30-gauge needle directly through the intact skin between the L5 and L6 vertebrae. Animals received either control serum or anti-VR1 serum (1:30,000–1:3000) at a volume of 5 μl/mouse 60 min before the tail-flick test.

2.4. Drugs

Streptozotocin was purchased from Sigma (St. Louis, MO). Antiserum to vanilloid VR1 receptor was obtained from Neuromics (MN, USA). This rabbit anti-vanilloid VR1 receptor serum cross-reacted with vanilloid VR1 receptor in mice. Control serum was collected from the same species of rabbits. Anti-vanilloid VR1 receptor serum and control serum were dissolved in normal saline.

2.5. Data analysis

All data are expressed as the mean ± S.E. The statistical significance of differences between groups was assessed with an analysis of variance (ANOVA) followed by the Bonferroni test (comparison among multiple groups).

3. Results

As shown in Fig. 1, in non-diabetic mice, a bulb voltage of 35 V did not cause a tail-flick response within the 30-s limit. However, when the voltage of the bulb was increased to 50 V, the mean tail-flick latency was significantly less than 30 s (Fig. 2). On the other hand, the heat intensity at a bulb voltage of 35 V did evoke a tail-flick response in diabetic mice, indicating that diabetic mice exhibit thermal allodynia (Fig. 1). Furthermore, the tail-flick latency at a bulb voltage of 50 V was shorter than that in non-diabetic mice, indicating that diabetic mice exhibit thermal hyperalgesia (Fig. 2).

I.t. pretreatment with anti-vanilloid VR1 receptor serum (1:30,000 ~ 1:3000) did not affect the tail-flick latency at a bulb voltage of 35 V in non-diabetic mice (Fig. 1). In contrast, the tail-flick latency at a bulb voltage of 35 V in diabetic mice was increased by i.t. pretreatment with anti-

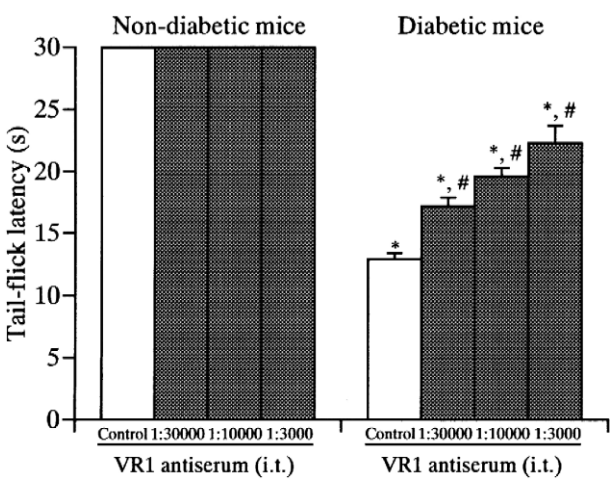

Fig. 1. Effect of anti-vanilloid VR1 receptor serum on the tail-flick latencies at a bulb voltage of 35 V in non-diabetic and diabetic mice. Anti-vanilloid VR1 receptor serum (VR1 antiserum, 1:30,000, 1:10,000 and 1:3000, hatched column) and control serum (open column) were injected i.t. 60 min before testing. Each point represents the mean with S.E. for 10 mice in each group. $^{*}P < 0.05$ compared with the respective non-diabetic group. $\#P < 0.05$ compared with the respective control serum-treated group.

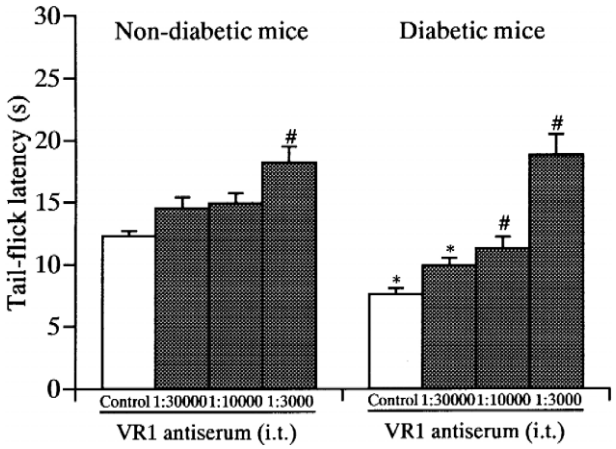

Fig. 2. Effect of anti-vanilloid VR1 receptor serum on the tail-flick latencies at a bulb voltage of 50 V in non-diabetic and diabetic mice. Anti-vanilloid VR1 receptor serum (VR1 antiserum, 1:30,000, 1:10,000 and 1:3000, hatched column) and control serum (open column) were injected i.t. 60 min before testing. Each point represents the mean with S.E. for 10 mice in each group. $^{*}P < 0.05$ compared with the respective non-diabetic group. $\#P < 0.05$ compared with the respective control serum-treated group.

vanilloid VR1 receptor serum in a concentration-dependent manner (1:30,000–1:3000) (Fig. 1).

On the other hand, as shown in Fig. 2, i.t. injection of 1:3000 diluted anti-vanilloid VR1 receptor serum resulted in a significant increase in the tail-flick latency at a bulb voltage of 50 V in non-diabetic, whereas 1:10,000 and 1:30,000 diluted anti-vanilloid VR1 receptor serum had no such effect. In diabetic mice, i.t. pretreatment with anti-vanilloid VR1 receptor serum at dilutions of 1:30,000, 1:10,000 and 1:3000 increased the tail-flick latency at a bulb voltage of 50 V in a concentration-dependent manner (Fig. 2).

4. Discussion

In the present study, diabetic mice showed thermal allodynia and hyperalgesia in the tail-flick test. These results are consistent with our previous observations that streptozotocin-induced diabetic mice showed thermal allodynia and hyperalgesia in the tail-flick test (Ohsawa and Kamei, 1999a,b; Kamei and Zushida, 2000).

In the present study, we observed that i.t. pretreatment with an anti-vanilloid VR1 receptor serum at 1:3000 produced significant antinociception in the tail-flick test using a bulb voltage of 50 V in both non-diabetic and diabetic mice. Furthermore, when diabetic mice were pretreated with anti-vanilloid VR1 receptor serum at 1:10,000, which had no effect on the tail-flick latency in non-diabetic mice, there was no significant difference between the tail-flick latencies at a bulb voltage of 50 V in diabetic and non-diabetic mice. These results indicate that anti-vanilloid VR1 receptor serum has an anti-hyperalgesic effect in diabetic mice.

Tail heating at a bulb voltage of 50 V increased the skin surface temperature of non-diabetic mice and diabetic mice to 45.7 ± 1.2°C and 44.6 ± 1.7°C, respectively. Since tail heating at a bulb voltage of 50 V produced a tail-flick response in non-diabetic mice and this response was significantly reduced by pretreatment with anti-vanilloid VR1 receptor serum, it is possible that heating at a bulb voltage of 50 V could activate vanilloid VR1 receptor. In contrast, heating at a bulb voltage of 35 V increased skin surface temperature of non-diabetic mice and diabetic mice to 42.4 ± 0.8°C and 35.2 ± 1.7°C, respectively. In non-diabetic mice, a bulb voltage of 35 V did not cause a tail-flick response within the 30-s limit. Thus, the heat intensity at a bulb voltage of 35 V may not have activated vanilloid VR1 receptor in non-diabetic mice. Interestingly, however, we observed that the heat intensity at a bulb voltage of 35 V did evoke a tail-flick response in diabetic mice. Furthermore, the tail flick response at a bulb voltage of 35V in diabetic mice was reduced by i.t. pretreatment with anti-vanilloid VR1 receptor serum in a concentration-dependent manner. These results indicate that the heat intensity at a bulb voltage of 35 V, which may not activate vanilloid VR1 receptor in non-diabetic mice, is sufficient to activate vanilloid VR1 receptor in diabetic mice. Thus, it seems likely that thermal hyperalgesia and allodynia in diabetic mice may be due to enhancement of the activation of vanilloid VR1 receptors in the spinal cord. We previously observed that the tail-flick latencies after heating at 35 and 50 V in diabetic mice were increased by i.t. pretreatment with a protein kinase C inhibitor, calphostin C, but not with a protein kinase A inhibitor, KT5720 ((8*R*, 9*S*, 11*S*)-(−)-9-hydroxy-9-*n*-hexyloxy-carbonyl-8-methyl-2, 3, 9, 20-tetrahydro-8, 11-epoxy-1*H*, 8*H*, 11*H*-2, 7*b*, 11*a*-triaqzadibenzo[*a*, *g*]cycloocta[cde]-trinden-1-one) (Ohsawa and Kamei, 1999a). Furthermore, in non-diabetic mice, tail-flick latencies were not affected by pretreatment with either a protein kinase C inhibitor or a protein kinase A inhibitor (Ohsawa and Kamei, 1999a). We also observed that tail-flick latencies at bulb voltages of 35 and 50 V were decreased by i.t. pretreatment with a protein kinase C activator, phorbol 12,13-dibutyrate, in non-diabetic mice, but not in diabetic mice (Ohsawa and Kamei, 1999a). Based on these results, we proposed that thermal hyperalgesia and allodynia in diabetic mice may be due to the activation of protein kinase C in the spinal cord (Ohsawa and Kamei, 1999a). Along these lines, Premkumar and Ahern (2000) demonstrated that activation of protein kinase C induces vanilloid VR1 receptor-gated channel activity in *Xenopus laevis* oocytes transfected with vanilloid VR1 receptors and in native vanilloid VR1 receptor from sensory neurons at room temperature in the absence of any other agonist. They also reported that treatment with phorbol ester to activate protein kinase C induced a vanilloid VR1 receptor-sensitive Ca^{2+} rise in sensory neurons (Pre-

mkumar and Ahern, 2000). Thus, it is possible that the increased protein kinase C activity in diabetic mice may be linked to the sensitization of vanilloid VR1 receptors.

In conclusion, the thermal hyperalgesia and allodynia seen in diabetic mice may be due, at least in part, to the sensitization of vanilloid VR1 receptors in primary sensory neurons in the spinal cord.

References

Calcutt, N.A., Chaplan, S.R., 1997. Spinal pharmacology of tactile allodynia in diabetic rats. Br. J. Pharmacol. 122, 1478–1482.

Caterina, M.J., Schumacher, M.S., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Caterina, M.J., Rosen, T.A., Tominaga, M., Brake, D., Julius, D., 1999. A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 398, 436–441.

De Castro, F., Silos-Santiago, I., Lopez de Armentia, M., Barbacid, M., Belmonte, C., 1998. Corneal innervation and sensitivity to noxious stimuli in trkA knockout mice. Eur. J. Neurosci. 10, 146–152.

Garcia-Hirschfeld, J., Lopez-Briones, L.G., Belmonte, C., Valdeolmillos, M., 1995. Intracellular free calcium responses to protons and capsaicin in cultured trigeminal neurons. Neuroscience 67, 235–243.

Helliwell, R.J., McLatchie, L.M., Clarke, M., Winter, J., Bevan, S., McIntyre, P., 1998. Capsaicin sensitivity is associated with the expression of the vanilloid (capsaicin) receptor (VR1) mRNA in adult rat sensory ganglia. Neurosci. Lett. 250, 177–180.

Hylden, J.L., Wilcox, G.L., 1980. Intrathecal morphine in mice: a new technique. Eur. J. Pharmacol. 67, 313–316.

Kamei, J., Zushida, K., 2000. Effect of mexiletine on thermal allodynia and hyperalgesia in diabetic mice. Jpn. J. Pharmacol. 84, 89–92.

Kamei, J., Ohhashi, Y., Aoki, T., Kasuya, Y., 1991. Streptozotocin-induced diabetes in mice reduces the nociceptive threshold, as recognized after application of noxious mechanical stimuli but not for thermal stimuli. Pharmacol. Biochem. Behav. 39, 541–544.

Ohsawa, M., Kamei, J., 1999a. Possible involvement of spinal protein kinase C in the thermal allodynia and hyperalgesia in diabetic mice. Eur. J. Pharmacol. 372, 221–228.

Ohsawa, M., Kamei, J., 1999b. Role of intracellular calcium in thermal allodynia and hyperalgesia in diabetic mice. Brain Res. 833, 278–281.

Premkumar, L.S., Ahern, G.P., 2000. Induction of vanilloid receptor channel activity by protein kinase C. Nature 408, 985–990.

Steen, K.H., Reeh, P.W., Anton, F., Handwerker, H.O., 1992. Protons selectively induce lasting excitation and sensitization to mechanical stimulation of nociceptors in rat skin, in vitro. J. Neurosci. 12, 86–95.

Tominaga, M., Caterina, M.J., Malmberg, A.B., Rosen, T.A., Gilbert, H., Skinner, K., Raumann, B.E., Basbaum, A.I., Julius, D., 1998. The cloned capsaicin receptor integrates multiple pain-modulating stimuli. Neuron 21, 531–543.